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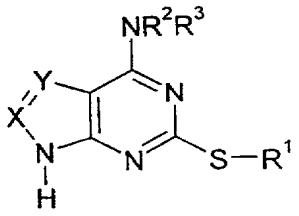
(54) Abstract Title
Pharmaceutically active pyrimidine derivatives

(57) A compound of formula (I) or a pharmaceutically acceptable salt or solvate thereof:



(I)

wherein R^1, R^2, R^3, X and Y are as defined in the specification, is useful for treating a chemokine mediated disease, eg. an inflammatory disease such as psoriasis.

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NOVEL COMPOUNDS

The present invention relates to pyrimidine compounds, processes and intermediates used in their preparation, pharmaceutical compositions containing them and their use in therapy.

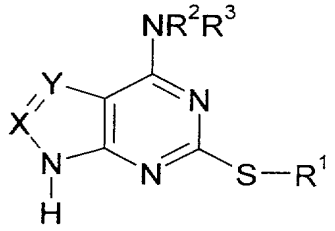
Chemokines play an important role in immune and inflammatory responses in various diseases and disorders, including asthma and allergic diseases, as well as autoimmune pathologies such as rheumatoid arthritis and atherosclerosis. These small secreted molecules are a growing superfamily of 8-14 kDa proteins characterised by a conserved four cysteine motif. The chemokine superfamily can be divided into two main groups exhibiting characteristic structural motifs, the Cys-X-Cys (C-X-C) and Cys-Cys (C-C) families. These are distinguished on the basis of a single amino acid insertion between the NH-proximal pair of cysteine residues and sequence similarity.

The C-X-C chemokines include several potent chemoattractants and activators of neutrophils such as interleukin-8 (IL-8) and neutrophil-activating peptide 2 (NAP-2).

The C-C chemokines include potent chemoattractants of monocytes and lymphocytes but not neutrophils such as human monocyte chemotactic proteins 1-3 (MCP-1, MCP-2 and MCP-3), RANTES (Regulated on Activation, Normal T Expressed and Secreted), eotaxin and the macrophage inflammatory proteins 1α and 1β (MIP-1α and MIP-1β).

Studies have demonstrated that the actions of the chemokines are mediated by subfamilies of G protein-coupled receptors, among which are the receptors designated CCR1, CCR2, CCR2A, CCR2B, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, CXCR1, CXCR2, CXCR3 and CXCR4. These receptors represent good targets for drug development since agents which modulate these receptors would be useful in the treatment of disorders and diseases such as those mentioned above.

In accordance with the present invention, there is therefore provided compounds of formula (I) or a pharmaceutically acceptable salts or solvates thereof:



(I)

in which:

R^1 represents an optionally branched C_1-C_4 alkyl group which terminates in an aryl or heteroaryl group each of which can be optionally substituted by one or more substituents independently selected from halogen atoms, cyano, nitro, $-OR^4$, $-NR^5R^6$, $-CONR^5R^6$, $-COOR^7$, $-NR^8COR^9$, $-SR^9$, $-SO_2R^9$, $-SO_2NR^5R^6$, $-NR^8SO_2R^9$, C_1-C_6 alkyl or trifluoromethyl groups and which may optionally contain one or more atoms independently selected from, O, NR^5, or S.

R^2 represents an optionally branched C_1-C_8 alkyl group terminating in an OH substituent which may be optionally substituted by one or more substituent groups independently selected from $-OR^4$, $-NR^5R^6$ $-CONR^5R^6$, $-NR^8COR^9$, $-SR^9$, $-SO_2R^9$, $-SO_2NR^5R^6$, $-NR^8SO_2R^9$,

R^3 represents hydrogen or C_1-C_6 alkyl optionally substituted by one or more substituent groups independently selected from halogen atoms, phenyl, $-OR^{10}$ and $-NR^{11}R^{12}$,

R^4 and R^5 independently represent a hydrogen atom or a C_1-C_6 alkyl or phenyl group the latter two of which may be optionally substituted by one or more substituent groups independently selected from halogen atoms, phenyl, $-OR^{13}$ and $-NR^{14}R^{15}$, $-CONR^{14}R^{15}$, $-NR^{14}COR^{15}$, $-SO_2NR^{14}R^{15}$, $NR^{14}SO_2R^{15}$

or

R^4 and R^5 together with the nitrogen atom to which they are attached form a 4- to 7-membered saturated heterocyclic ring system optionally comprising a further heteroatom selected from oxygen and nitrogen atoms, which ring system may be optionally substituted by one or more substituent groups independently selected from phenyl, $-OR^{13}$, $-COOR^{13}$, $-NR^{14}R^{15}$, $-CONR^{14}R^{15}$, $-NR^{14}COR^{15}$, $-SO_2NR^{14}R^{15}$, $-NR^{14}SO_2R^{15}$ or C_1-C_6 alkyl, itself optionally substituted by one or more substituents independently selected from halogen atoms and $-NR^{14}R^{15}$ and $-OR^{16}$ groups,

R^9 represents a hydrogen atom, a C_1-C_6 alkyl group or a phenyl group, each of which may be optionally substituted by one or more substituent groups independently selected from halogen atoms, phenyl, -OR^{16} and -$NR^{14}R^{15}$,

X represents a nitrogen atom, CH or $CNR^{14}R^{15}$;

Y represents a nitrogen atom or CH; and

each of R^7, R^8, R^{10}, R^{11}, R^{12}, R^{13}, R^{14} R^{15}, and R^{16} independently represents a hydrogen atom or a C_1-C_6, alkyl, or a phenyl group,

provided that when Y is CH, then X is not CH or C-$NR^{14}R^{15}$ and when Y is N then X is not $CNR^{14}R^{15}$.

In the context of the present specification, unless otherwise indicated, an alkyl or alkenyl group or an alkyl or alkenyl moiety in a substituent group may be linear or branched.

Aryl groups include phenyl and naphthyl. Heteroaryl is defined as a 5- or 6-membered aromatic ring optionally containing one or more heteroatoms selected from N, S, O. Examples include pyridine, pyrimidine, thiazole, oxazole, pyrazole, imidazole, furan.

Certain compounds of formula (I) are capable of existing in stereoisomeric forms. It will be understood that the invention encompasses all geometric and optical isomers of the compounds of formula (I) and mixtures thereof including racemates. Tautomers and mixtures thereof also form an aspect of the present invention.

In formula (I) above, the group R^1 represents an optionally branched C_1-C_4 alkyl group which terminates in an aryl or heteroaryl group each of which can be optionally substituted by one or more substituents independently selected from halogen atoms, cyano, nitro, -OR^4, -NR^5R^6, -$CONR^5R^6$, -$COOR^7$, -NR^8COR^9, -SR^9, -SO_2R^9, -$SO_2NR^5R^6$, -$NR^8SO_2R^9$, C_1-C_6 alkyl or trifluoromethyl groups and which may optionally contain one or more atoms independently selected from, O, NR^5, or S. Particularly advantageous compounds of formula (I) are those in which R^1 represents an optionally substituted benzyl group. More preferably R^1 represents benzyl or benzyl substituted by one or more halogen atoms, in particular benzyl substituted by two fluoro atoms.

Suitably R^2 represents an optionally branched C_1-C_8 alkyl group terminating in an OH substituent which may be optionally substituted by one or more substituent groups independently selected from $-OR^4$, $-NR^5R^6$ $-CONR^5R^6$, $-NR^8COR^9$, $-SR^9$, $-SO_2R^9$, $-SO_2NR^5R^6$, $-NR^8SO_2R^9$.

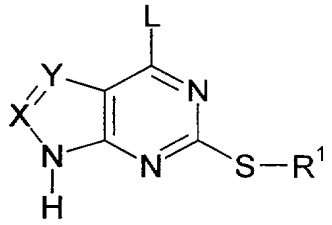
Preferably R^2 is $CH(CH_3)CH_2OH$, $CH(Et)CH_2OH$ or $C(CH_3)_2CH_2OH$. Most preferably one of R^2 and R^3 is hydrogen and the other is $CH(CH_3)CH_2OH$.

Suitably R^3 represents hydrogen or C_1-C_6 alkyl optionally substituted by one or more substituent groups independently selected from halogen atoms, phenyl, $-OR^{10}$ and $-NR^{11}R^{12}$. Preferably R^3 represents hydrogen.

Suitably X represents a nitrogen atom, CH or $C-NR^{15}R^{16}$. Preferably X represents a nitrogen atom or CH group. Suitably Y represents a nitrogen atom or CH group.

Particularly preferred compounds of the invention include:
(2*R*)-2-[2-[(phenylmethyl)thio]-(1*H*-purin-6-yl)amino]-1-propanol,
(2*R*)-2-[[6-[[(2,3-Difluorophenyl)methyl]thio]-1*H*-pyrazolo[3,4-*d*] pyrimidin-4-yl] amino]-1-propanol, and
2-Methyl-2-[(5-phenylmethylthio-3*H*-[1,2,3]-triazolo[4,5-*d*]pyrimidin-7-yl)amino]-1-propanol
and pharmaceutically acceptable salts and solvates thereof.

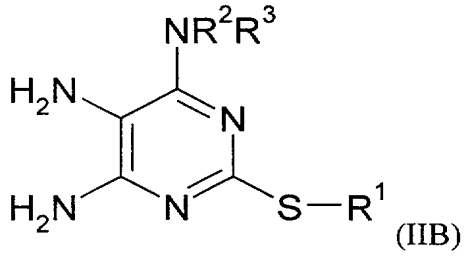
According to the current invention there is also provided a process for the preparation of a compound of formula (I) which comprises:
(a) treatment of a compound of formula (IIA):



(IIA)

where X, Y and R^1 are as defined in formula (I) and L is a leaving group with an amine HNR^2R^3, or

(b) for compounds where X and Y are both N, reacting a compound of general formula (IIB):



(IIB)

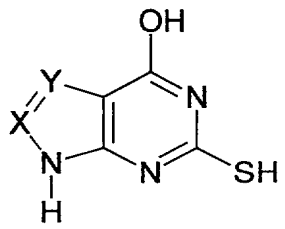
wherein R^1, R^2 and R^3 are as defined in formula (I) with a nitrosating agent, and optionally thereafter (a) or (b) forming a pharmaceutically acceptable salt.

The reaction of compounds of formula (IIA) with an amine HNR^2R^3 can be carried out in a solvent such as N-methyl-pyrrolidinone at a temperature between 0°C and 150°C. Suitable leaving groups L include halogen, especially chloro.

The reaction of compounds of formula (IIB) with a nitrosating agent can be carried out in a solvent such as acetonitrile at reflux. Suitable nitrosating agents include isoamyl nitrite.

Compounds of formula (IIA) where L is halogen can be prepared by treating a compound of formula (IIA) where X, Y and R^1 are as defined in formula (I) and L is an hydroxyl group with a halogenating agent such as phosphorus oxychloride. The reaction may be carried out at reflux in the presence of dimethylaniline.

Compounds of formula (IIA) where L is hydroxyl group are suitably prepared by reacting a compound of formula (III):

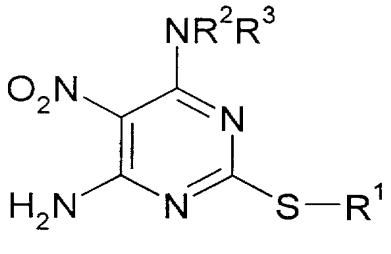


(III)

with a compound of formula R^1L^1 where R^1 is as defined above and L^1 is a leaving group such as bromide in the presence of a base such as sodium hydroxide. The reaction may be carried out in aqueous ethanol at room temperature.

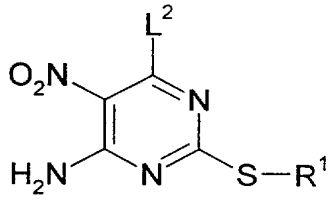
Compounds of formula (III) are either commercially available or can be prepared using standard chemistry.

Compounds of formula (IIB) where R^1, R^2 and R^3 are as defined in formula (I) are suitably prepared by reacting a compound of formula (IV) with a reducing agent such as sodium hydrosulphite. The reaction may be carried out in aqueous dioxane at a temperature between 20°C and 100°C.



(IV)

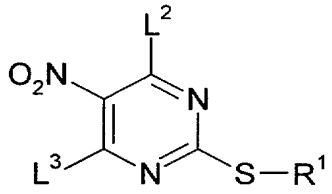
Compounds of formula (IV) where R^1, R^2 and R^3 are as defined in formula (I) are suitably prepared by reacting a compound of formula (V):



(V)

wherein L^2 represents a leaving group such as a halogen atom (e.g. chlorine) and R^1 is as defined in formula (I) with an amine HNR^2R^3. The reaction may be carried out in a solvent such as dimethylformamide at a temperature between 0°C and 150°C.

Compounds of formula (V) where L^2 is a leaving group and R^1 is as defined in formula (I) are suitably prepared by reacting a compound of formula (VI):

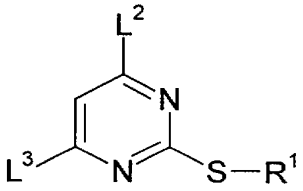


(VI)

wherein L^3 represents a leaving group such as halogen (e.g. chlorine) and R^1 is as defined above with ammonia. The reaction may be carried out in a solvent such as diethyl ether at room temperature.

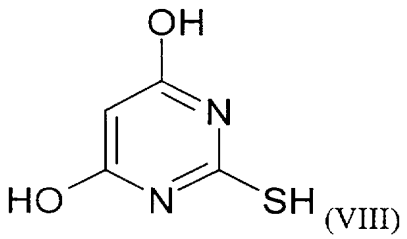
Compounds of formula (VI) where R^1 is as defined in formula (I) and L^2 and L^3 are halogen may be prepared by treating a compound of formula (VI) where R^1 is as defined in formula (I) and L^2 and L^3 are hydroxyl groups with a halogenating agent such as phosphorus oxychloride. The reaction may be carried out in a solvent such as toluene at 100°C.

Compounds of formula (VI) where R^1 is as defined in formula (I) and L^2 and L^3 are hydroxyl groups may be prepared by treating a compound of formula (VII) where R^1 is as defined in formula (I) and L^2 and L^3 are hydroxyl groups with a nitrating agent such as concentrated nitric acid. The reaction may be carried out in a solvent such as glacial acetic acid at a temperature between 0°C and 100°C.



(VII)

Compounds of formula (VII) where R^1 is as defined in formula (I) and L^2 and L^3 are hydroxyl groups are suitably prepared by reacting a compound of formula (VIII):



(VIII)

with a compound of formula R^1X where R^1 is as defined above and X is a leaving group such as bromide in the presence of a base such as sodium hydroxide. The reaction may be carried out in aqueous NMP at room temperature.

Compounds of formula (VII) are commercially available.

It will be appreciated by those skilled in the art that in the processes of the present invention certain functional groups such as hydroxyl or amino groups in the starting reagents or intermediate compounds may need to be protected by protecting groups. Thus, the preparation of the compounds of formula (I) may involve, at an appropriate stage, the removal of one or more protecting groups.

The protection and deprotection of functional groups is fully described in 'Protective Groups in Organic Chemistry', edited by J. W. F. McOmie, Plenum Press (1973), and 'Protective Groups in Organic Synthesis', 2nd edition, T. W. Greene & P. G. M. Wuts, Wiley–Interscience (1991).

Novel intermediate compounds form a further aspect of the invention.

The compounds of formula (I) above may be converted to a pharmaceutically acceptable salt or solvate thereof, preferably an acid addition salt such as a hydrochloride, hydrobromide, phosphate, acetate, fumarate, maleate, tartrate, citrate, oxalate, methanesulphonate or *p*-toluenesulphonate.

The compounds of formula (I) have activity as pharmaceuticals, in particular as modulators of chemokine receptor (especially CXCR2) activity, and may be used in the treatment (therapeutic or prophylactic) of conditions/diseases in human and non-human animals which are exacerbated or caused by excessive or unregulated production of chemokines. Examples of such conditions/diseases include:

(1) **(the respiratory tract)** obstructive airways diseases including chronic obstructive pulmonary disease (COPD) such as irreversible COPD; asthma, such as bronchial, allergic, intrinsic, extrinsic and dust asthma, particularly chronic or inveterate asthma (e.g. late asthma and airways hyper-responsiveness); bronchitis; acute, allergic, atrophic rhinitis and chronic rhinitis including rhinitis caseosa, hypertrophic rhinitis, rhinitis purulenta, rhinitis sicca and rhinitis medicamentosa; membranous rhinitis including croupous, fibrinous and pseudomembranous rhinitis and scrofoulous rhinitis; seasonal rhinitis including rhinitis nervosa (hay fever) and vasomotor rhinitis; sarcoidosis, farmer's lung and related diseases, fibroid lung and idiopathic interstitial pneumonia;

(2) **(bone and joints)** rheumatoid arthritis, seronegative spondyloarthropathies (including ankylosing spondylitis, psoriatic arthritis and Reiter's disease), Behcet's disease, Sjogren's syndrome and systemic sclerosis;

(3) **(skin)** psoriasis, atopical dermatitis, contact dermatitis and other eczmatous dermitides, seborrhoetic dermatitis, Lichen planus, Pemphigus, bullous Pemphigus, Epidermolysis bullosa, urticaria, angiodermas, vasculitides, erythemas, cutaneous eosinophilias, uveitis, Alopecia areata and vernal conjunctivitis;

(4) **(gastrointestinal tract)** Coeliac disease, proctitis, eosinopilic gastro-enteritis, mastocytosis, Crohn's disease, ulcerative colitis, food-related allergies which have effects remote from the gut, e.g., migraine, rhinitis and eczema;

(5) **(other tissues and systemic disease)** multiple sclerosis, atherosclerosis, Acquired Immunodeficiency Syndrome (AIDS), lupus erythematosus, systemic lupus, erythematosus, Hashimoto's thyroiditis, myasthenia gravis, type I diabetes, nephrotic syndrome, eosinophilia fascitis, hyper IgE syndrome, lepromatous leprosy, sezary syndrome and idiopathic thrombocytopenia pupura;

(6) **(allograft rejection)** acute and chronic following, for example, transplantation of kidney, heart, liver, lung, bone marrow, skin and cornea; and chronic graft versus host disease;

(7) cancers, especially non-small cell lung cancer (NSCLC) and squamous sarcoma;

(8) diseases in which angiogenesis is associated with raised CXCR2 chemokine levels (e.g. NSCLC); and

(9) cystic fibrosis, stroke, re-perfusion injury in the heart, brain, peripheral limbs and sepsis.

Thus, the present invention provides a compound of formula (I), or a pharmaceutically-acceptable salt or solvate thereof, as hereinbefore defined for use in therapy.

In a further aspect, the present invention provides the use of a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as hereinbefore defined in the manufacture of a medicament for use in therapy.

In a still further aspect, the present invention provides the use of a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as hereinbefore defined in the manufacture of a medicament for the treatment of human diseases or conditions in which modulation of chemokine receptor activity is beneficial.

In the context of the present specification, the term "therapy" also includes "prophylaxis" unless there are specific indications to the contrary. The terms "therapeutic" and "therapeutically" should be construed accordingly.

The invention still further provides a method of treating a chemokine mediated disease wherein the chemokine binds to a CXCR2 receptor, which comprises administering to a patient a therapeutically effective amount of a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as hereinbefore defined.

The invention also provides a method of treating an inflammatory disease, especially psoriasis, in a patient suffering from, or at risk of, said disease, which comprises administering to the patient a therapeutically effective amount of a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as hereinbefore defined.

For the above-mentioned therapeutic uses the dosage administered will, of course, vary with the compound employed, the mode of administration, the treatment desired and the disorder indicated.

The compounds of formula (I) and pharmaceutically acceptable salts and solvates thereof may be used on their own but will generally be administered in the form of a pharmaceutical composition in which the formula (I) compound/salt/solvate (active ingredient) is in association with a pharmaceutically acceptable adjuvant, diluent or carrier. Depending on the mode of administration, the pharmaceutical composition will preferably comprise from 0.05 to 99 %w (per cent by weight), more preferably from 0.05 to 80 %w, still more preferably from 0.10 to 70 %w, and even more preferably from 0.10 to 50 %w, of active ingredient, all percentages by weight being based on total composition.

The present invention also provides a pharmaceutical composition comprising a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as hereinbefore defined, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

The invention further provides a process for the preparation of a pharmaceutical composition of the invention which comprises mixing a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as hereinbefore defined, with a pharmaceutically acceptable adjuvant, diluent or carrier.

The pharmaceutical compositions may be administered topically (e.g. to the lung and/or airways or to the skin) in the form of solutions, suspensions, heptafluoroalkane aerosols and dry powder formulations; or systemically, e.g. by oral administration in the form of tablets, capsules, syrups, powders or granules, or by parenteral administration in the form of solutions or suspensions, or by subcutaneous administration or by rectal administration in the form of suppositories or transdermally.

The invention will now be further illustrated by reference to the following examples. In the examples the Nuclear Magnetic Resonance (NMR) spectra were measured on a Varian Unity Inova 300 or 400 MHz spectrometer and the Mass Spectrometry (MS) spectra measured on a Finnigan Mat SSQ7000 or Micromass Platform spectrometer. Where necessary, the reactions were performed under an inert atmosphere of either nitrogen or argon. Chromatography was generally performed using Matrex Silica 60® (35-70 micron) or Prolabo Silica gel 60® (35-70 micron) suitable for flash silica gel chromatography. High pressure liquid chromatography purification was performed using either a Waters Micromass LCZ with a Waters 600 pump controller, Waters 2487 detector and Gilson FC024 fraction collector or a Waters Delta Prep 4000. The abbreviations m.p. and DMSO used in the examples stand for melting point and dimethyl sulphoxide respectively.

Example 1
(2*R*)-2-[2-[(phenylmethyl)thio]-(1*H*-purin-6-yl)amino]-1-propanol

a) 1,7-Dihydro-2-[(phenylmethyl)thio]-6*H*-purin-6-one

6-hydroxy-2-mercaptopurine (2g) was dissolved in a mixture of ethanol (10ml), 2N aqueous sodium hydroxide (11ml) and water (15ml). Under ice cooling and with stirring, benzyl bromide (2.1ml) was added to the solution and it was left stirring at room temperature for 30min. The solution was then cooled and neutralised with 2N HCl and diluted with 30ml isohexane. The resulting precipitate was filtered, washed with ethyl acetate and dried over P_2O_5 at 50°C for 2 hours to afford the subtitled product as a white solid (3.11g).

m.p. 230-231°C
MS: ADCI(+ve) 259 (M+1)
^{1}H NMR: δ (DMSO) 4.44 (s,2H), 7.24-7.46 (m,6H), 8.03 (s,1H).

b) 6-chloro-2-[(phenylmethyl)thio]-1*H*-purine

The product of step (a) (4.4g) was placed in a three-necked flask with nitrogen flowing gently through one neck, a septum on the second and a condenser on the third. Phosphorus oxychloride (60ml) was added to form a solid suspension, then *N*, *N*-dimethylaniline (4ml) was added via syringe and the mixture was heated to 120°C in an oil bath for 2 hours. It was then allowed to cool overnight. The resulting mixture was poured on to ice with stirring and the ice was allowed to melt. The crude product was filtered off and washed with water and then purified using preparative HPLC to afford the subtitled product as a white solid (2.61g).

m.p.175-176°C
MS: APCI(+ve) 277.5 (M+1)
^{1}H NMR: δ (DMSO) 4.45 (s,2H), 7.23-7.49 (m,5H), 8.54 (s,1H) 13.78 (br s,1H)

(2*R*)-2-[2-[(phenylmethyl)thio]-(1*H*-purin-6-yl)amino]-1-propanol

The product of step (b) (500mg) was mixed with D-alaninol (1ml) in N-methylpyrrolidinone (15ml), and the mixture was heated to 100°C for 11 hours. The reaction mixture was cooled to room temperature, diluted with ethyl acetate (200ml), washed with brine (100ml), and the organic phase dried over $MgSO_4$, filtered and evaporated. The crude product was purified by preparative HPLC to give the title product as a white solid (224mg).

m.p. 208-209°C
MS: APCI(+ve) 316 (M+1)
^{1}H NMR: δ (DMSO) 1.16 (d,3H), 3.32-3.54 (m,2H), 4.37 (m,3H), 4.77 (br s,1H), 7.19-7.44 (m,6H), 12.70 (br s,1H), 7.98 (s, 1H).

Example 2
(2*R*)-2-[[6-[[(2,3-Difluorophenyl)methyl]thio]-1*H*-pyrazolo[3,4-*d*] pyrimidin-4-yl] amino]-1-propanol

a) 6-[[(2,3-Difluorophenyl)methyl]thio]-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ol

6-mercapto-1*H*-pyrazolo [3,4-*d*] pyrimidin-4-ol (5g) was added to a solid suspension of 60% NaH (1.42g) in dry DMF (50ml) and left to stir for 1hour. 2,3-Difluorobenzyl bromide was then added slowly and the reaction mixture stirred for 1-2 hours. The resulting mixture was poured in to water whereupon the sub-titled product precipitated out. It was then filtered, washed with water and dried over P_2O_5 at 50°C for 10hrs (4.46g).

MS: APCI (+ve) 295 (M+1)
^{1}H NMR: δ (DMSO) 4.52 (s,2H), 7.14-7.21 (m,1H), 7.32-7.43 (m,2H), 8.11 (br s,1H), 12.34 (br s,1H), 13.64 (br s,1H).

b) 4-Chloro-6-[[(2,3-difluorophenyl)methyl]thio]-1*H*-pyrazolo[3,4-*d*]pyrimidine

The product of step (a) (4.4g) was placed in a three-necked flask with nitrogen flowing gently through one neck, a septum on the second and a condenser on the third. Phosphorus oxychloride (60ml) was added to form a solid suspension, then *N*, *N*-dimethylaniline (6.0ml) added dropwise by syringe and the mixture was heated to 120°C in an oil bath for 2 hours. It was then allowed to cool overnight. The resulting mixture was poured on to ice with stirring and the ice was allowed to melt. The crude product was filtered off and

washed with water and then purified using column chromatography eluting with DCM then 20% ethyl acetate/80% DCM. The appropriate fractions were evaporated to give the sub-titled product as a white solid (2.12g).

m.p. 191-192 °C
MS: APCI (+ve) 313.5 (M+1)
^{1}H NMR: δ (DMSO) 4.55 (s,2H), 7.13-7.21 (m,1H), 7.31-7.46 (m,2H), 8.34 (s,1H), 14.31 (s,1H).

c) (2*R*)-2-[[6-[[(2,3-difluorophenyl)methyl]thio]-1*H*-pyrazolo[3,4-*d*] pyrimidin-4-yl] amino]-1-propanol

The product of step (b) (250mg) was mixed with D-alaninol (1ml) in NMP (4ml), and the mixture was heated to 100°C for 11 hours. The reaction mixture was extracted with ethyl acetate (2x100ml) and brine (100ml), and the organic phase was dried over $MgSO_4$, filtered and evaporated. The crude product was purified by preparative HPLC to give the title product as a white solid (160mg).

m.p. 205-206 °C
MS: APCI (+ve) 352 (M+1)
^{1}H NMR: δ (DMSO) 1.17 (d,3H), 3.38-3.52 (m,2H), 4.81 (t,1H), 7.12-7.17 (m,1H), 7.29-7.41 (m,2H), 8.06 (s/d,2H), 13.21 (s,1H).

Example 3

2-Methyl-2-[(5-phenylmethylthio-3*H*-[1,2,3]-triazolo[4,5-*d*]pyrimidin-7-yl)amino]-1-propanol

a) 2-Phenylmethylthio-4,6-dihydroxypyrimidine

To a solution of 4,6-dihydroxy-2-mercaptopyrimidine (100 g) in 5M NaOH (360 ml) was added first NMP (200 ml), then benzyl bromide (90 ml) dropwise over 2 hours. The reaction was stirred at room temperature for 24 hours, then acidified to pH 2 with conc HCl (100 ml) added dropwise over 2 hours at –5°C to give a pink precipitate. This was isolated by decanting off the solution followed by trituration with diethyl ether (1 litre) to give the subtitled product as a white powder after filtration and drying (210 g).

m.p. 220-250°C (dec)
MS: APCI (-ve) 233 (M-H)
^{1}H NMR: δ (DMSO) 11.76 (1H, br s), 7.45 (2H, d), 7.26 (3H, m), 5.18 (1H, s), 4.38 (2H, s).

b) 2-Phenylmethylthio-4,6-dihydroxy-5-nitropyrimidine

The product of step (a) (2 g) was added to a mixture of glacial acetic acid (50 ml) and concentrated nitric acid (20 ml) and the reaction mixture heated to 50°C. A further 28 g of the product of step (a) was added in portions over 2 hours whilst maintaining the reaction temperature between 50 and 60°C. After stirring the reaction mixture for a further 1 hour at 50°C it was poured onto crushed ice and the subtitled product isolated by filtration as a yellow solid (12.3 g).

^{1}H NMR: δ (DMSO) 7.48-7.19 (5H, m), 4.47 (2H, s).

c) 2-Phenylmethylthio-4,6-dichloro-5-nitropyrimidine

A suspension of the product of step (b) (59.2 g) in a mixture of $POCl_3$ (100 ml) and toluene (400 ml) was heated to 80°C. A solution of 1-methylimidazole (16.9 ml) in toluene (200 ml) was added dropwise over 1 hour and the reaction mixture then heated at 100°C for 24 hours. After cooling to room temperature, the solvent was removed by evaporation and water (2 litres) cautiously added to the residue. The mixture was extracted with dichloromethane (4x500 ml) and the combined organic extracts dried over $MgSO_4$, filtered and evaporated to give a brown tar. This was purified by column chromatography, eluting with 10% dichloromethane in isohexane, to afford the subtitled product as a yellow solid (29.7 g).

MS: APCI (-ve) 315 (M-H)
^{1}H NMR: δ (DMSO) 7.43-7.24 (5H, m), 4.40 (2H, s).

d) 4-Amino-2-phenylmethylthio-6-chloro-5-nitropyrimidine

To a solution of the product of step (c) (1 g) in diethyl ether (20 ml) was added first *N,N'*-diisopropylethylamine (1.1 ml) followed by a solution of methanolic 7N NH3 (1.2 ml) in diethyl ether (10 ml) dropwise over 30 minutes. The reaction mixture was stirred at room

temperature for 24 hours, then acidified to pH 2 by the addition of conc HCl (5 ml). The organic phase was separated, dried over $MgSO_4$, filtered and evaporated to afford the subtitled product as a yellow solid (0.58 g).

^{1}H NMR: δ (DMSO) 7.40-7.25 (5H, m), 4.36 (2H, s).

e) 2-[(6-Amino-5-nitro-2-[(phenylmethyl)thio]-4-pyrimidinyl]amino-2-methyl-1-propanol

2-Amino-2-methyl-1-propanol (0.09 ml) was added to a solution of the product of step (d) (100 mg) in DMF (7 ml) and the reaction mixture stirred at room temperature for 24 hours. The solvent was removed by evaporation and the residue purified by column chromatography, eluting with 30% ethyl acetate in isohexane, to afford the subtitled product as a yellow solid (106 mg).

MS: APCI (+ve) 349 (M^+)
^{1}H NMR: δ (DMSO) 9.67 (1H, s), 8.69 (1H, s), 8.60 (1H, s), 7.43-7.24 (5H, m), 5.21 (1H, t), 4.37 (2H, s), 3.45 (2H, d), 1.37 (6H).

f) 2-{[(5,6-diamino-2-phenylmethylthio)-4-pyrimidinyl]amino}-2-methyl-1-propanol

Sodium hydrosulphite (9.3 g) was added in portions to a solution of the product of step (e) in 1,4-dioxane (60 ml) at 55°C. The reaction mixture was stirred vigorously at this temperature whilst water (50 ml) was added dropwise over 30 minutes. The 1,4-dioxane was removed by evaporation giving a white precipitate which was isolated by filtration. This was purified by column chromatography, eluting with 25% dichloromethane in ethyl acetate, to afford the subtitled product as a white solid (0.67 g).

^{1}H NMR: δ (DMSO) 7.40-7.20 (5H, m), 5.77 (2H, s), 5.35 (1H, s), 5.06 (1H, t), 4.25 (2H, s), 3.48 (2H, s), 1.29 (6H, s).

g) 2-Methyl-2-[(5-phenylmethylthio-3*H*-[1,2,3]-triazolo[4,5-*d*]pyrimidin-7-yl)amino]-1-propanol

Isoamyl nitrite (0.41 ml) was added to a suspension of the product of step (f) in in acetonitrile (50 ml) and the reaction mixture heated at reflux for 30 minutes. The solvent

was evaporated and the residue purified by column chromatography, eluting with 3% methanol in dichloromethane, to afford the title compound as a white solid which was further purified by recrystallization from ethyl acetate (43 mg).

m.p. 191°C
MS: APCI (+ve) 330 (M^+)
^{1}H NMR: δ (DMSO) 7.43-7.21 (5H, m), 4.87 (1H, br s), 4.42 (2H, s), 3.60 (2H, s), 1.28 (6H, s).

Pharmacological Data

Ligand Binding Assay

[^{125}I]IL-8 (human, recombinant) was purchased from Amersham, U.K. with a specific activity of 2,000Ci/mmol. All other chemicals were of analytical grade. High levels of hrCXCR2 were expressed in HEK 293 cells (human embryo kidney 293 cells ECACC No. 85120602) (Lee *et al.* (1992) *J. Biol. Chem.* **267** pp16283-16291). hrCXCR2 cDNA was amplified and cloned from human neutrophil mRNA. The DNA was cloned into PCRScript (Stratagene) and clones were identified using DNA. The coding sequence was sub-cloned into the eukaryotic expression vector RcCMV (Invitrogen). Plasmid DNA was prepared using Quiagen Megaprep 2500 and transfected into HEK 293 cells using Lipofectamine reagent (Gibco BRL). Cells of the highest expressing clone were harvested in phosphate-buffered saline containing 0.2%(w/v) ethylenediaminetetraacetic acid (EDTA) and centrifuged (200g, 5min.). The cell pellet was resuspended in ice cold homogenisation buffer [10mM HEPES (pH 7.4), 1mM dithiothreitol, 1mM EDTA and a panel of protease inhibitors (1mM phenyl methyl sulphonyl fluoride, 2μg/ml soybean trypsin inhibitor, 3mM benzamidine, 0.5μg/ml leupeptin and 100μg/ml bacitracin)] and the cells left to swell for 10 minutes. The cell preparation was disrupted using a hand held glass mortar/PTFE pestle homogeniser and cell membranes harvested by centrifugation (45 minutes, 100,000g, 4°C). The membrane preparation was stored at -70°C in homogenisation buffer supplemented with Tyrode's salt solution (137mM NaCl, 2.7mM KCl, 0.4mM NaH_2PO_4), 0.1%(w/v) gelatin and 10%(v/v) glycerol.

All assays were performed in a 96-well MultiScreen 0.45μm filtration plates (Millipore, U.K.). Each assay contained ~50pM [^{125}I]IL-8 and membranes (equivalent to ~200,000 cells) in assay buffer [Tyrode's salt solution supplemented with 10mM HEPES (pH 7.4), 1.8mM $CaCl_2$, 1mM $MgCl_2$, 0.125mg/ml bacitracin and 0.1%(w/v) gelatin]. In addition, a compound of formula (I) according to the Examples was pre-dissolved in DMSO and

added to reach a final concentration of 1%(v/v) DMSO. The assay was initiated with the addition of membranes and after 1.5 hours at room temperature the membranes were harvested by filtration using a Millipore MultiScreen vacuum manifold and washed twice with assay buffer (without bacitracin). The backing plate was removed from the MultiScreen plate assembly, the filters dried at room temperature, punched out and then counted on a Cobra γ-counter.

The compounds of formula (I) according to the Examples were found to have IC_{50} values of less than (<) 10μM.

Intracellular Calcium Mobilisation Assay

Human neutrophils were prepared from EDTA-treated peripheral blood, as previously described (Baly *et al.* (1997) Methods in Enzymology 287 pp70-72), in storage buffer [Tyrode's salt solution (137mM NaCl, 2.7mM KCl, 0.4mM NaH_2PO_4) supplemented with 5.7mM glucose and 10mM HEPES (pH 7.4)].

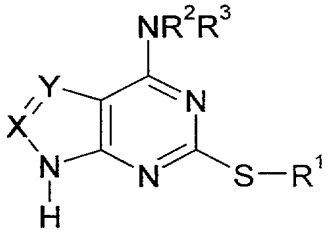
The chemokine GROα (human, recombinant) was purchased from R&D Systems (Abingdon, U.K.). All other chemicals were of analytical grade. Changes in intracellular free calcium were measured fluorometrically by loading neutrophils with the calcium sensitive fluorescent dye, fluo-3, as described previously (Merritt *et al.* (1990) Biochem. J. 269, pp513-519). Cells were loaded for 1 hour at 37°C in loading buffer (storage buffer with 0.1%(w/v) gelatin) containing 5μM fluo-3 AM ester, washed with loading buffer and then resuspended in Tyrode's salt solution supplemented with 5.7mM glucose, 0.1%(w/v) bovine serum albumin (BSA), 1.8mM $CaCl_2$ and 1mM $MgCl_2$. The cells were pipetted into black walled, clear bottom, 96 well micro plates (Costar, Boston, U.S.A.) and centrifuged (200g, 5 minutes, room temperature).

A compound of formula (I) according to the Examples was pre-dissolved in DMSO and added to a final concentration of 0.1%(v/v) DMSO. Assays were initiated by the addition of an A_{50} concentration of GROα and the transient increase in fluo-3 fluorescence (λ_{Ex} =490nm and λ_{Em} = 520nm) monitored using a FLIPR (Fluorometric Imaging Plate Reader, Molecular Devices, Sunnyvale, U.S.A.).

The compounds of formula (I) according to the Examples were tested and found to be antagonists of the CXCR2 receptor in human neutrophils.

CLAIMS

1. A compound of formula (I) or a pharmaceutically acceptable salt or solvate thereof:



(I)

in which

R^1 represents an optionally branched C_1-C_4 alkyl group which terminates in an aryl or heteroaryl group each of which can be optionally substituted by one or more substituents independently selected from halogen atoms, cyano, nitro, $-OR^4$, $-NR^5R^6$, $-CONR^5R^6$, $-COOR^7$, $-NR^8COR^9$, $-SR^9$, $-SO_2R^9$, $-SO_2NR^5R^6$, $-NR^8SO_2R^9$, C_1-C_6 alkyl or trifluoromethyl groups and which may optionally contain one or more atoms independently selected from, O, NR^5, or S.

R^2 represents an optionally branched C_1-C_8 alkyl group terminating in an OH substituent which may be optionally substituted by one or more substituent groups independently selected from $-OR^4$, $-NR^5R^6$ $-CONR^5R^6$, $-NR^8COR^9$, $-SR^9$, $-SO_2R^9$, $-SO_2NR^5R^6$, $-NR^8SO_2R^9$,

R^3 represents hydrogen or C_1-C_6 alkyl optionally substituted by one or more substituent groups independently selected from halogen atoms, phenyl, $-OR^{10}$ and $-NR^{11}R^{12}$

R^4 and R^5 independently represent a hydrogen atom or a C_1-C_6 alkyl or phenyl group the latter two of which may be optionally substituted by one or more substituent groups independently selected from halogen atoms, phenyl, $-OR^{13}$ and $-NR^{14}R^{15}$, $-CONR^{14}R^{15}$, $-NR^{14}COR^{15}$, $-SO_2NR^{14}R^{15}$, $NR^{14}SO_2R^{15}$

or

R^4 and R^5 together with the nitrogen atom to which they are attached form a 4- to 7-membered saturated heterocyclic ring system optionally comprising a further heteroatom

selected from oxygen and nitrogen atoms, which ring system may be optionally substituted by one or more substituent groups independently selected from phenyl, $-OR^{13}$, $-COOR^{13}$, $-NR^{14}R^{15}$, $-CONR^{14}R^{15}$, $-NR^{14}COR^{15}$, $-SO_2NR^{14}R^{15}$, $-NR^{14}SO_2R^{15}$ or C_1-C_6 alkyl, itself optionally substituted by one or more substituents independently selected from halogen atoms and $-NR^{14}R^{15}$ and $-OR^{16}$ groups,

R^9 represents a hydrogen atom, a C_1-C_6 alkyl group, or a phenyl group, each of which may be optionally substituted by one or more substituent groups independently selected from halogen atoms, phenyl, $-OR^{16}$ and $-NR^{14}R^{15}$,

X represents a nitrogen atom, CH or $CNR^{14}R^{15}$;

Y represents a nitrogen atom or CH; and

each of R^7, R^8, R^{10}, R^{11}, R^{12}, R^{13}, R^{14} R^{15}, and R^{16} independently represents a hydrogen atom or a C_1-C_6, alkyl, or a phenyl group,

provided that when Y is CH, then X is not CH or C-$NR^{14}R^{15}$ and when Y is N then X is not $CNR^{14}R^{15}$.

2. A compound according to claim 1, wherein R^1 represents an optionally substituted benzyl group.

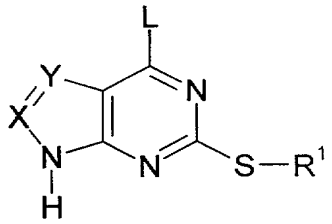
3. A compound according to claim 1 or claim 2, where R^2 is $CH(CH_3)CH_2OH$, $CH(Et)CH_2OH$ or $C(CH_3)_2CH_2OH$.

4. A compound according to claim 1 selected from:
(2*R*)-2-[2-[(phenylmethyl)thio]-(1*H*-purin-6-yl)amino]-1-propanol,
(2*R*)-2-[[6-[[(2,3-Difluorophenyl)methyl]thio]-1*H*-pyrazolo[3,4-*d*] pyrimidin-4-yl] amino]-1-propanol,
2-Methyl-2-[(5-benzylthio-3*H*-[1,2,3]-triazolo[4,5-*d*]pyrimidin-7-yl)amino]-1-propanol

and pharmaceutically acceptable salts and solvates thereof.

5. A process for the preparation of a compound of formula (I) as defined in claim 1 which comprises:

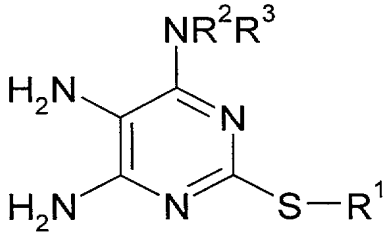
(a) treatment of a compound of formula (IIA):



(IIA)

where X, Y and R^1 are as defined in formula (I) and L is a leaving group with an amine HNR^2R^3, or

(b) for compounds where X and Y are both N, reacting a compound of general formula (IIB):



(IIB)

wherein R^1, R^2 and R^3 are as defined in formula (I) with a nitrosating agent, and optionally thereafter (a) or (b) forming a pharmaceutically acceptable salt.

6. An intermediate compound of formula (II) as defined in claim 5.

7. A pharmaceutical composition comprising a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as claimed in any one of claims 1 to 4 in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

8. A process for the preparation of a pharmaceutical composition as claimed in claim 7 which comprises mixing a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as claimed in any one of claims 1 to 6 with a pharmaceutically acceptable adjuvant, diluent or carrier.

9. A compound of formula (I), or a pharmaceutically-acceptable salt or solvate thereof, as claimed in any one of claims 1 to 4 for use in therapy.

10. Use of a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as claimed in any one of claims 1 to 4 in the manufacture of a medicament for use in therapy.

11. A method of treating a chemokine mediated disease wherein the chemokine binds to a CXCR2 receptor, which comprises administering to a patient a therapeutically effective amount of a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as claimed in any one of claims 1 to 4.

12. A method of treating an inflammatory disease in a patient suffering from, or at risk of, said disease, which comprises administering to the patient a therapeutically effective amount of a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as claimed in any one of claims 1 to 4.

13. A method according to claim 12, wherein the disease is psoriasis.

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Application No: GB 0003022.1
Claims searched: 1-5 and 7-13

Examiner: Peter Davey
Date of search: 18 May 2000

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.R): C2C (CRM)

Int Cl (Ed.7):

Other: Online: CAS ONLINE

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
	NONE	

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.